

POST-PLEISTOCENE GEOGRAPHIC VARIATION IN *JUNIPERUS COMMUNIS* IN NORTH AMERICA

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ABSTRACT

Plants of *Juniperus communis* L. var. *communis*, *J. c.* var. *depressa* Pursh, *J. c.* var. *megistocarpa* Fern. & St. John, *J. c.* var. *saxatilis* Pall. were sampled and DNA fingerprinting (RAPDs, Random Amplified Polymorphic DNAs) was performed. Based on 100 RAPD bands, *J. communis* var. *communis* and *J. c.* var. *saxatilis* from the eastern hemisphere were clearly separated from *J. communis* in North America. Populations referred to as var. *saxatilis* from the Pacific northwest did not show alliances with authentic var. *saxatilis* from Europe. However, plants with short, curved leaves, and having a white stomatal band about twice as wide as the green margins are allied with *J. c.* var. *jackii* Rehrd. Geographic variation in North American populations of *J. communis* revealed the southern Appalachian Mountains appear to have been a refugium during the late Pleistocene (Wisconsin). Plants from the isolated Mt. Charleston, Nevada were very differentiated, reflecting a long period of genetic isolation. The populations at Banff and Canmore, Alberta were somewhat intermediate between *J. c.* var. *depressa* and *J. c.* var. *jackii* from Queen Charlotte Island.

KEY WORDS: *Juniperus communis*, Cupressaceae, geographic variation, Pleistocene

The genus *Juniperus* consists of approximately 68 species and 36 varieties (Adams, 2004). All the taxa grow in the northern hemispheres, except *J. procera* Hochst. ex Endl. which grows along the rift mountains in east Africa, thence into the southern hemisphere

(Adams, Demeke and Abulfatih 1993), and some of the Mediterranean *Juniperus* species such as *J. oxycedrus* L., *J. phoenicea* L., and *J. thurifera* L. that grow in the mountains of the northernmost part of Africa (Morocco, Algeria).

Juniperus communis is the only *Juniperus* species that occurs in both hemispheres. In North America, *J. communis* has been treated (Adams, 2004) as composed of as many as four varieties (Fig. 1). Most



Figure 1. Distribution of *J. communis* var. *depressa*, *J. c.* var. *jackii*, *J. c.* var. *megistocarpa*, and putative *J. c.* var. *saxatilis* in North America.

of the present distribution was covered with ice during the late Pleistocene (Wisconsin), so recolonization of these areas has been recent (10-12,000 y). A number of isolated populations in the southern portion of the distributions may have served as refugia, as these areas were likely never glaciated (Fig. 1). Adams (2004) considered *J. c.* var. *jackii* to be a part of *J. c.* var. *saxatilis* in the Pacific northwest (Fig. 1).

Figure 2, from a study of Arctic populations of *J. communis* (Adams et al., 2003), revealed that these Arctic populations clustered by continent with the populations in Greenland and Iceland showing the

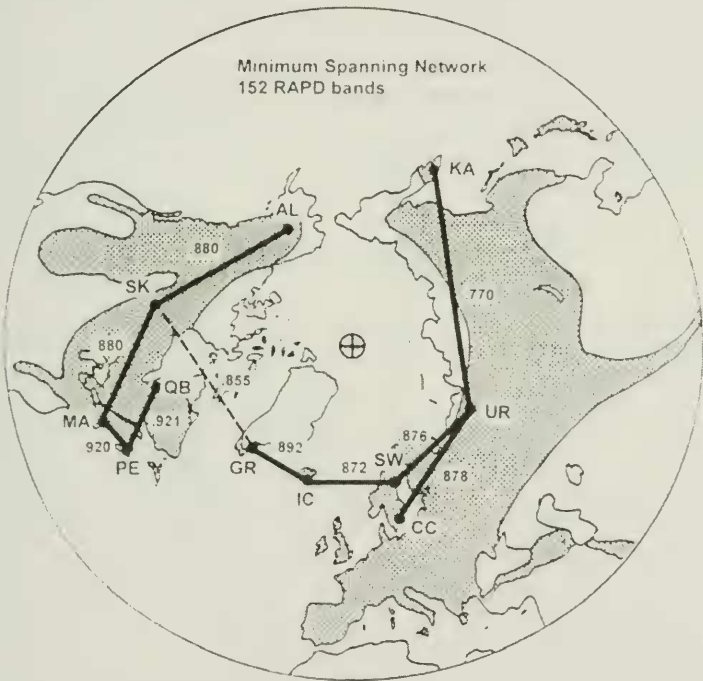


Figure 2. Minimum spanning network showing that all the North American *J. communis* populations link together and all the *J. communis* populations from the e. hemisphere link together.

highest affinities to populations from Europe, not those from North American. The North American populations were all *J. c.* var. *depressa*, whereas the eastern hemisphere populations included *J. c.* var. *communis* (CC), *J. c.* var. *saxatilis* (GR, IC, SW, UR, KA). Adams et al. (2003) concluded that the post-Pleistocene populations on Greenland and Iceland came from Europe and not North America.

Analysis of the currently named *Juniperus communis* varieties (Adams and Pandey, 2003), resolved these taxa (Fig. 3) into six major groups: *J. communis* from Europe and central Asia (*J. communis* L. var. *communis*, *J. c.* var. *depressa* Pursh, N. America; *J. c.* var. *saxatilis* Pall.); *J. c.* var. *megistocarpa* Fern. & St. John, Quebec; *J. c.* var. *nipponica* (Maxim.) E. H. Wilson, Japan; and putative *J. c.* var.

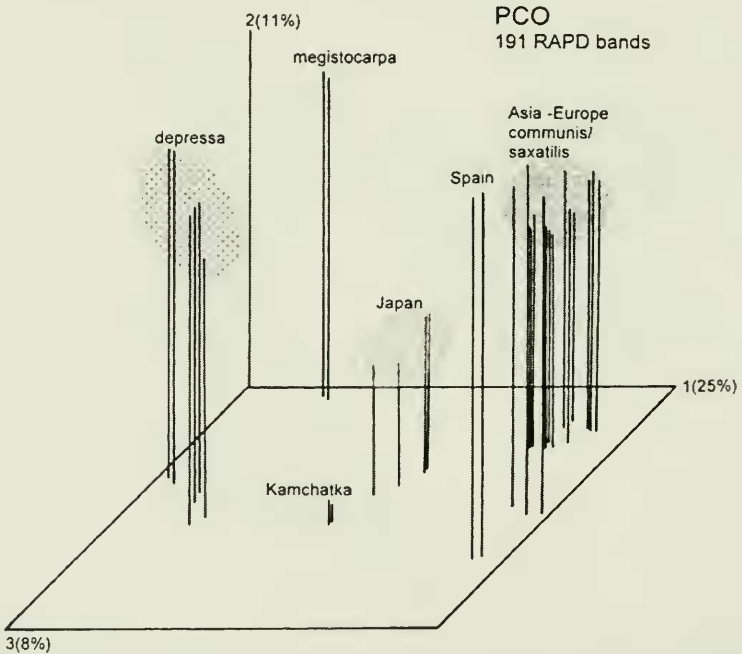


Figure 3. PCO of *J. communis* varieties (from Adams and Pandey, 2003). See text for discussion.

saxatilis, Kamchatka, Russia. However, Adams and Pandey (2003) did not include *J. c. var. jackii*, nor putative *J. c. var. saxatilis* from the Pacific northwest, USA/ Canada in their analysis.

Ashworth, et al. (1999, 2001) used DNA fingerprinting to examine *J. communis* plants identified as *J. c. var. depressa*, *J. c. var. jackii* Rehder, *J. c. var. montana* Aiton (= *J. c. var. saxatilis* Pall. see Adams, 2004) collected from California, Oregon, Nevada or Utah in the southwest and west coast of the United States. They did not get a clear pattern separating these taxa, and concluded that their samples represent a single varietal taxon. However, it not clear if they utilized population samples to remove spurious variation in RAPD bands.

In the present study, we have collected additional samples of putative *J. c. var. saxatilis* from the Pacific northwest, *J. c. var. jackii* from nw California and *J. c. var. depressa* from the southernmost locations in North America (Mt. Charleston, Nevada and Mt. Satula, North Carolina).

MATERIALS AND METHODS

Specimens used in this study: *J. communis* var. *communis* : Adams 7846, 7848, Stockholm, Sweden; *J. c. var. depressa*: Adams 7582, 7582, Denali National Park, Alaska, USA; Adams 7094, 7095, on granite bluff, Neimembian Lake, Saskatchewan, Canada; Adams 10366, 10367, Hudson Bay, Quebec, Canada (ex N. Dignard); Adams 10317, 10318, on glacial till, Canmore, Alberta, Canada; Adams 10282, 10283, Mt. Charleston, Clark Co. NV, USA; Adams 10225, 10226, on granite, Mt. Satula, Macon Co., NC, USA; *J. c. var. jackii*: Adams 10287, 10288, serpentine, Del Norte Co., CA, USA; *J. c. var. megistocarpa*, Adams 8575, 8576, Magdalene Islands, Quebec, Canada (ME); *J. c. var. saxatilis*: Adams 10467, 10467 (Mtns.), 10481, 10482 (coastal) Norway (ex. J. Karlsen); Putative *J. c. var. saxatilis*: Adams 9181, 9182 (ex. J. W. Leverenz), Esso, Kamchatka Peninsula, Russia; Adams 10304, 10305, Queen Charlotte Isl., BC; Adams 10300, 10301, on volcanic rock, Mt. Hood, Wasco Co., Oregon, USA; Adams 10328, 10329, possible hybrids, Hoodoos, Banff, BC, Canada. Voucher specimens are deposited at the Baylor University herbarium (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia, CA). The RAPD analyses follow that of Adams and Demeke (1993). Ten-mer primers were purchased from the University of British Colombia (5'-3'): 134, AAC ACA CGA G; 153, GAG TCA CGA G; 184, CAA ACG GAC C; 212, GCT GCG TGA C; 218, CTC AGC CCA G; 239, CTG AAG CGG A; 249, GCA TCT ACC G; 250, CGA CAG TCC C; 268, AGG CCG CTT A; 338, CTG TGG CGG T; 346, TAG GCG AAC G; 347, TTG CTT GGC G; 375, CCG GAC ACG A; 431, CTG CGG GTC A; 478, CGA GCT GGT C.

PCR stock solutions (Taq, primer, and buffer) were made in bulk so that all the PCR reaction tubes for a primer were prepared using the same bulk stock. This is a critical factor for minimizing variation in band intensities from sample to sample (see Adams, Flournoy and Pandey, 1998, for protocols to minimize PCR band variation). PCR was performed in a volume of 15 µl containing 50 mM KCl, 10 mM Tris-HCl (pH 9), 2.0 mM MgCl₂, and 0.1% Triton X-100, 0.2 mM of each dNTPs, 0.36 µM primers, 0.3 ng genomic DNA, 15 ng BSA and 0.6 unit of Taq DNA polymerase (Promega). A negative control PCR tube containing all components, but no genomic DNA, was run with each primer to check for contamination. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). Samples were run in duplicate to insure reproducibility (Adams, Flournoy and Pandey, 1998). A temperature profile was obtained for each well of the thermocycler to be sure that no variation existed among wells in the heating/ cooling block. The thermal cycle used was: 94° C (1.5 min) for initial strand separation, then 40 cycles of 40° C (2 min), 72° C (2 min), 91° C (1 min). Two additional steps were used: 40° C (2 min) and 72° C (5 min) for final extension. The temperature inside a PCR tube containing 15 µl buffer was monitored with a temperature probe, quantitated and printed for each step for each of the 40 cycles for every PCR run (Adams, Flournoy and Pandey, 1998) to insure that each cycle met temperature specifications and that each PCR run was exactly the same. Amplification products were analyzed by electrophoresis on 1.5% agarose gels, 75V, 55 min, and detected by staining with ethidium bromide. The gels were

photographed over UV light using Polaroid film 667 and scanned to digital images. The digital images were size normalized in reference to pGem® DNA size markers before band scoring. Bands were scored as present (1) and absent (0). Bands that were inconsistent in replicate analyses were not scored.

Associational measures were computed using absolute character state differences (Manhattan metric), divided by the maximum observed value for that character over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis (PCO) was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). It should be noted that problems of homology of RAPD DNA bands on agarose gels can be significant (Rieseberg, 1996), but these errors can be accounted for using multivariate statistical methods (PCO) (see Adams and Rieseberg, 1998). A minimum spanning diagram was constructed by selecting the nearest neighbor for each taxon from the pair-wise similarity matrix, then connecting those nearest neighbors as nodes in a network (Adams, et al. 2003).

RESULTS AND DISCUSSION

The major trend (figure 4) among the taxa is the separation of the eastern hemisphere plants (*J. communis* var. *communis*, *J. c.* var. *saxatilis*, and putative *J. c.* var. *saxatilis*, Kamchatka) from the western hemisphere plants (*J. c.* var. *depressa*, *J. c.* var. *jackii*, *J. c.* var. *megistocarpa*, and putative var. *saxatilis*). The resolution (figure 4) of *J. c.* var. *jackii* (and plants from nearby Mt. Hood) is in contrast to the report by Ashworth, et al. (1999, 2001). The Banff, Alberta individuals (putative hybrids) are intermediate between the coastal, short, curved leaved plants (Queen Charlotte Islands plants, var. *jackii*) and *J. c.* var. *depressa* (figure 4). *Juniperus c.* var. *megistocarpa* is distinct from *J. c.* var. *depressa*.

The most interesting facet of this PCO is that putative *J. c.* var. *saxatilis* (Queen Charlotte Islands, Mt. Hood, OR, and *J. c.* var. *jackii* plants) do not cluster with *J. c.* var. *saxatilis* (Norway, mountain, figure 4). It appears that the short, curved leafed taxon from the Pacific northwest thence into Alaska is part of a variable taxon, *J. c.* var. *jackii*.

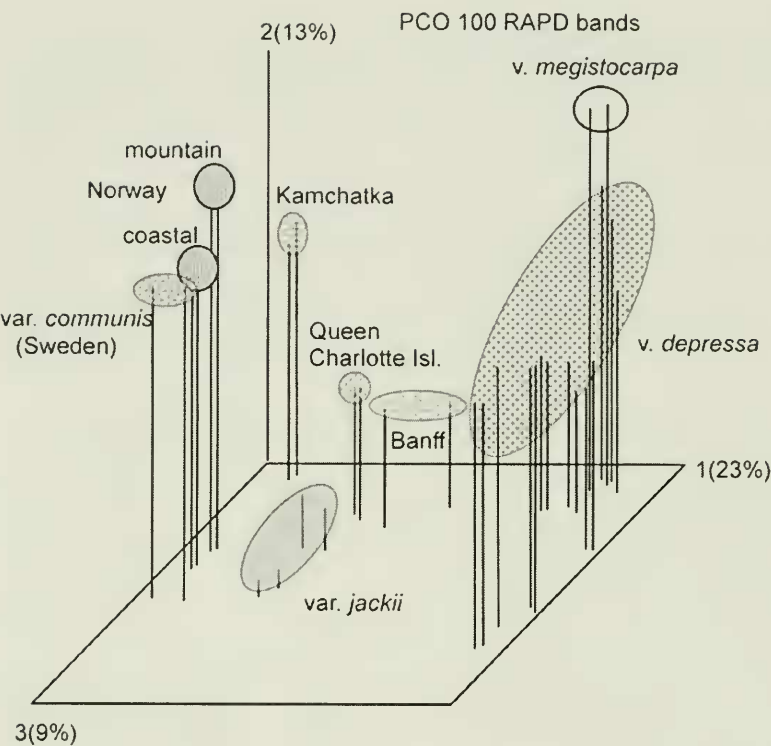


Figure 4. PCO based on 100 RAPD bands. See text for discussion.

To examine the geographic trends, the plants from the eastern hemisphere and the intermediate Banff individuals were removed from analysis and a new similarity matrix was constructed. Contouring the clustering is shown in figure 5. The major trend shows that three very geographically separated populations in Denali National Park, Alaska, (D), Hudson Bay, Quebec (H), and Mt. Satula, North Carolina (NC) are the least genetically differentiated. Certainly, there are numerous bridging populations between Alaska and Hudson Bay, but the North Carolina population is quite distant from adjacent populations.

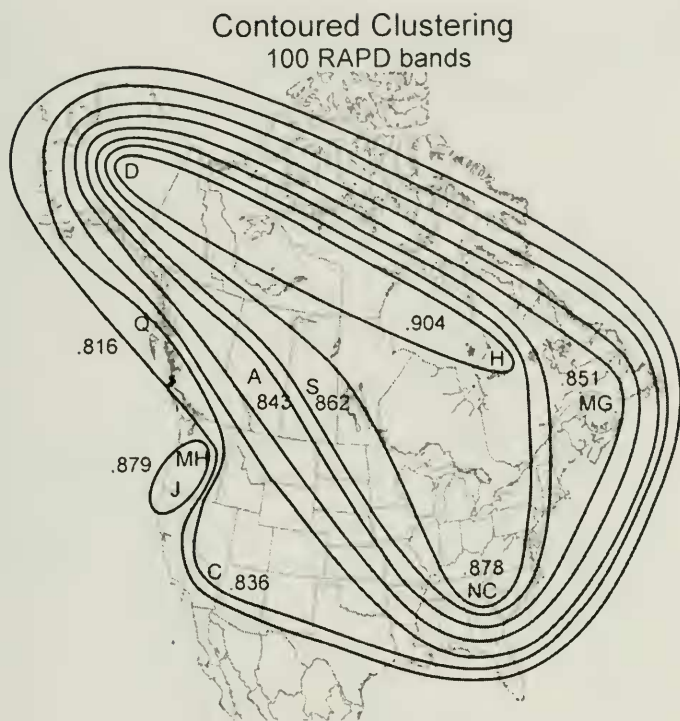


Figure 5. Contoured clustering based on 100 RAPD bands using minimum spanning values.

The second facet of the contoured clustering (figure 5) is that *J. c. var. jackii* and Mt. Hood, OR plants are divergent from the bulk of the North America *J. c. var. depressa* populations. The Queen Charlotte Islands (Q) are also quite divergent, but fail to link with the *J. c. var. jackii* group. It seems likely that all these infra-specific populations are interfertile and that introgression from *J. c. var. depressa* may be occurring into the Queen Charlotte Island plants. The Queen Charlotte Islands plants do, however, maintain short, curved leaves like *J. c. var. jackii*.

Another interesting trend is that *J. c. var. megistocarpa* (large fruited, common juniper on sand dunes, MG) is more similar (figure 5)

to most *J. c. var. depressa* populations than the Mt. Charleston (C) population is to *J. c. var. depressa*.

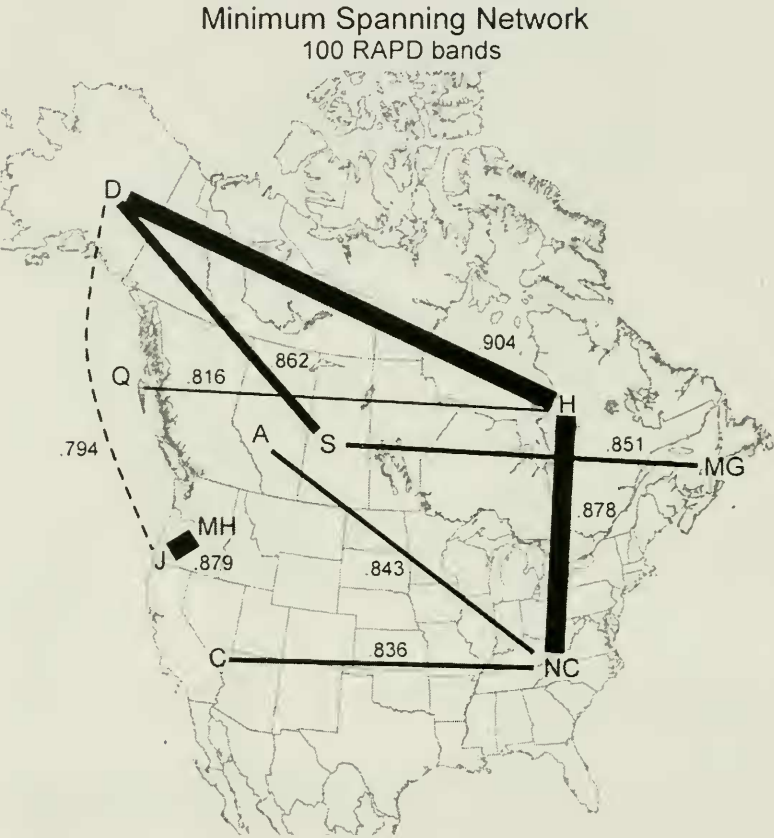


Figure 6. Minimum spanning network based on 100 RAPD bands. The line width is indicative of the similarity between populations.

Examination of the linkage among populations shows (figure 6) a strong north-south and southeast-northwest linkage. *Juniperus c. var. megistocarpa* (MG) links with *J. c. var. depressa* from Saskatchewan (S). The adjacent population of *J. c. var. depressa* in Alberta (A) links with the North Carolina (NC) population. The Queen

Charlotte Islands population (Q) links with the Hudson Bay (H) plants, which also have short, curved leaves. The leaf length and curvature may be somewhat environmentally induced in the colder locations. The *J. c. var. jackii* populations (J, MH) link at a lower level to the Denali, Alaska (A) plants.

It is possible that the Alaska (A) population was not glaciated during the Wisconsin (figure 7), but all the other northern populations were glaciated. Only the Alaska (D), Mt. Charleston (C) and North

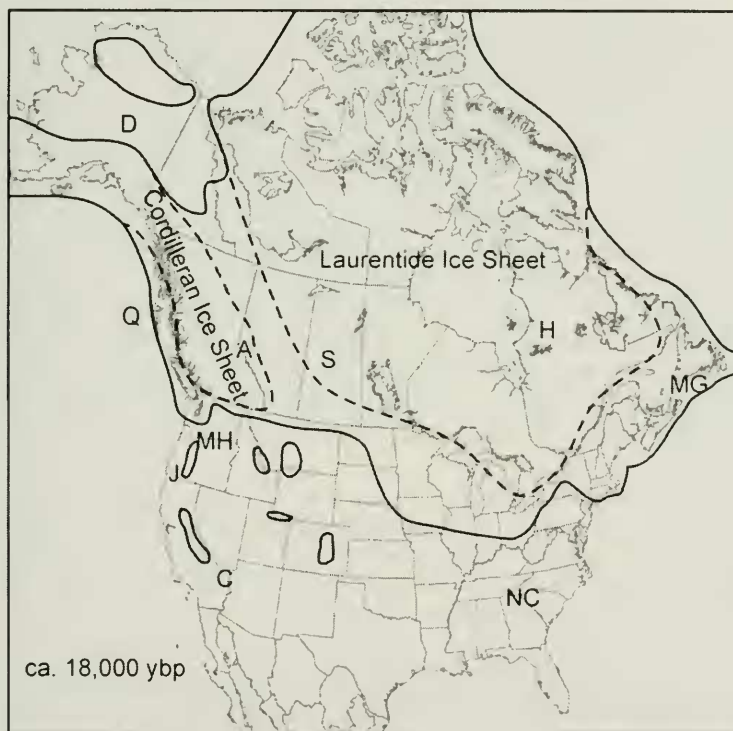


Figure 7. Maximal ice cover during the late Wisconsin. Notice that only the Alaska (D), Mt. Charleston (C) and North Carolina (NC) populations may have survived *in situ* or nearby.

Carolina (NC) populations may have survived during the Wisconsin. The *J. c.* var. *jackii* populations (J, MH) likely moved to lower elevations. However, the northwestern California population of *J. c.* var. *jackii* presently occurs on serpentine, so it seems unlikely that this edaphic type grew on serpentine at a lower elevation. *Juniperus* is well known to be very adaptive to edaphic conditions, so Wisconsin era genotypes may have merely invaded the largely open habitat on the serpentine of northwestern California and southwestern Oregon.

There seem to be four possible refugia during the Wisconsin: southern Appalachian Mts. (cf. NC); southern Rocky Mountains (cf. Mt. Charleston and Arizona/ New Mexico Mts.; central Sierra Nevada; and possibly an ice free corridor in central Alaska. It is easy to imagine that birds carried seeds from plants from the southern Appalachians northward into northern US and Canada. It appears more likely that the southern Appalachians were the source of germplasm in re-colonization of Canada than the southern Rocky Mountains. Notice, figure 6, that the linkage of Mt. Charleston (C) is to North Carolina (NC) rather than to Alberta (A) or Saskatchewan (S). The relictual status of the Alaska (D) population is uncertain. It does show strong linkage with both Hudson Bay and Saskatchewan populations (fig. 6) and it could have been the source of germplasm in recolonization of Canada. Conversely, the Alaskan population may have not survived the Wisconsin and it may have been re-colonized with seeds from central Canada.

The *J. c.* var. *jackii* group was distinct in this analysis; however, the addition of samples from central California near Mono Lake, CA, the Puget Sound/ Vancouver area, Idaho, and Alaska may change our concepts of the short, curved leafed *J. communis* group.

At present, it seems prudent to recognize three varieties of *J. communis* in North America: var. *depressa* with long, straight leaves, stomatal band width 1 to 1.5 x width of green side band (figure 8); var. *jackii* with short, curved leaves, stomatal band 2 x width of green side band from central California to Alaska (figure 8); and var. *megistocarpa*, with large female cones, restricted to sand dunes and rocky beaches in far ne Canada (figure 8).



Figure 8. Distribution of *J. communis* in North America. The range of the *J. c.* var. *jackii* genotype has not been verified except in nw California and w Oregon.

This analysis indicates that *J. c.* var. *saxatilis*, as known in Europe and the eastern hemisphere, is not represented in North America. It is not clear from this study if *J. c.* var. *jackii* is confined to n California and Oregon or is widespread from California to Alaska. The distribution of *J. c.* var. *jackii* (figure 8) is based on leaf morphology suggesting that a single variety exists in the northwestern US, western Canada and Alaska. Additional sampling and DNA analysis is needed to resolve this problem.

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